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THESIS FOR DOCTORAL DEGREE (Ph.D.)

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In the name of Allah, Most Gracious, Most Merciful

To my parents and family

ABSTRACT

Effective infection prevention and control hospital program is essential for prevention hospital associated infections (HAIs). Central line-associated bloodstream infection (CLABSI) is one of the major threating patients in intensive care units (ICUs). However, CLABSIs can be prevented through proper prevention measures and guidelines information resources are free available in many national and international professional health agencies.

Methicillin resistant *Staphylococcus aureus* (MRSA) is a major bacterial agent that causing community-and-hospital associated (CA/HA) infections and related to significant morbidity and mortality rates worldwide. Shigellosis is a highly infectious disease threating public health, especially with *Shigella sonnei* species in developed and developing countries. These two microorganisms have ability to develop resistance to many available antimicrobial agents and consequence successfully cloned and distributed globally.

In 2011, the annual risk assessment of King Abdul Aziz Specialist hospital in Taif of Saudi Arabia has reported high incidence rate of CLABSI and the characteristic susceptibility patterns of community-associated methicillin resistant *Staphylococcus aureus* (CA-MRSA) infections is different from the other locality in Saudi Arabia (data not published).

High-resolution melting (HRM) has been used for MRSA *spa* typing in clinical diagnostic laboratories for epidemiological purposes as accurate, rapid and cost effective scheme method. Rotor gene 6000-system is excellent fully equipped for real-time amplification for HRMA. It is possible to introduce a hypothesis of HRM is accrete and rapid of MRSA *spa*-typing and *S. sonnei* genotyping method for epidemiological purposes.

The hospital CLABSI prevention project was analyzed and extended to assess the susceptibility pattern, genotyping and virulence gene detection of MRSA infections in Saudi Arabia. Also, we tested the hypothesis of HRM genotyping by evaluating HRM of MRSA *spa* typing and identification of *S. sonnei* lineages and sub lineages using Rotor gene 6000-system among globally collected samples.

There was about 60% reduction of CLABSIs after implementation of Society for Healthcare Epidemiology of America/Infectious Diseases Society of America (SHEA/IDSA) practice guidelines. HRM can distinguishable identification of MRSA *spa* types as well as *S. sonnei* lineages and sub lineages. Most of MRSA *spa* types were unambiguously typed. All *S. sonnei* lineages and sub lineages were identified using HRM real-time PCR. Also, the reproducibility was assessed and results revealed the same. We observed high prevalence of panton valentine leukocidine (PVL) positive related to hospital associated MRSA infections (HA-MRSA) is Saudi Arabia.

The basic SHEA/IDSA practice recommendation is an effective prevention model for the reduction of CLABSI in the ICU. HRM-based is reproducible, simple, rapid and cost-effective for *spa*-typing and *S. sonnei* genotyping method. Emerging multi drug resistance S. *aureus* strains with PVL gene circulating within hospitals alarms the urgent need for continuous active surveillance and implementation prevention measures.

LIST OF SCIENTIFIC PAPERS

I. Waleed Mazi, Zikra Begum, Diaa Abdullah, Ahmed Hesham, Sami Maghari, Abdullah Assiri and Abiola Senok. Central line-associated bloodstream infection in a trauma intensive care unit: Impact of implementation of Society for Healthcare Epidemiology of America/Infectious Diseases Society of America practice guidelines. American Journal of Infection Control, 2014; 42:865-7.

II. Waleed Mazi, Jun Yu, Gunnar Sandström and Amir Saeed. Emerging PVL-positive and multidrug-resistant *Staphylococcus aureus* in hospital-associated infections in Taif, Saudi Arabia. (Manuscript).

III. Waleed Mazi, Vartul Sangal, Gunnar Sandström, Amir Saeed and Jun Yu. Evaluation of *spa*-typing of methicillin–resistant *Staphylococcus aureus* using high-resolution melting analysis. International Journal of Infectious Diseases, 2015; 38: 125-8.

IV. Waleed Mazi, Vartul Sangal, Amir Saeed, Gunnar Sandström, Francois-Xavier Weill and Jun Yu. Rapid genotyping of *Shigella sonnei* by use of multiplex high-resolution melting. Journal of Clinical Microbiology, 2015; 53:2389-1.

CONTENTS

LIST OF ABBREVIATIONS

Bp	Base pair
BSAC	British Society for Antimicrobial Chemotherapy
CA-MRSA	Community-associated methicillin resistant *Staphylococcus aureus*
CC	Clonal Complex
CDC	US Center for Disease Control and Prevention
CH-Taif	Children´s Hospital-Taif
CLABSI	Central line-associated bloodstream infection
E-CDC	European Centre for Disease Prevention and Control
HAI	Healthcare associated infection
HRM	High resolution melting
HA-MRSA	Hospital-associated methicillin resistant *Staphylococcus aureus*
ICU	Intensive Care Unit
IDSA	Infectious Diseases Society of America
INICC	International Nosocomial Infection Control Consortium
KAASH-Taif	King Abdul Aziz Specialist Hospital-Taif
KFH-Taif	King Faisal Hospital-Taif
MIC	Minimum inhibitory concentration
MLST	Multi locus sequence typing
MRSA	Methicillin resistant *Staphylococcus aureus*
NHSN	National Healthcare System Network
PCR	Polymerase chain reaction
PVL	Panton Valentine Leukocidine
SHEA	Society for Healthcare Epidemiology of America
SNP	Single nucleotide polymorphism
Spa	Staphylococcal protein A
ST	Sequence type
Tm	Melting temperature
WHO	World Health Organization

1 INTRODUCTION

Effective infection prevention and control programmes in hospitals are essential to reduce and/or control infection risk factors and to prevent infections whenever possible. These require involvement by all staff members, patients, family members, students and visitors in order to maintain a healthy and safe environment. Healthcare-associated infections (HAIs) are the most adverse event threatening hospital patient safety worldwide. Many factors contribute to the risk of hospital-acquired infections, including inadequate hand hygiene compliance, poor infrastructure, understaffing, overcrowding, inappropriate invasive devices and inappropriate antimicrobial management [1].

1.1 CENTRAL LINE-ASSOCIATED BLOODSTREAM INFECTION

Central line-associated bloodstream infection (CLABSI) is responsible for increased morbidity, mortality, extended duration of hospital stay and excess healthcare costs in intensive care units (ICUs) world-wide [2]. International Nosocomial Infection Control Consortium (INICC) is a multinational research network established to control and reduce HAIs at international level. It has reported that the incidence rate of CLABSI in ICUs in developing countries is three-fold higher than that in the United States [3]. CLABSI can be prevented through proper prevention measures, for which guidelines and information resources are freely available from many national and international professional health agencies. These include INICC, World Health Organization (WHO), European Centre for Disease Prevention and Control (ECDC), US Center for Disease Control and Prevention (CDC), Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). In the United States, the National Healthcare System Network (NHSN) was established in 2005 to collaborate with CDC on creation of infection surveillance systems using standardised methods and to categorise all infections using standard CDC definitions that include laboratory and clinical criteria. The CDC/NHSN system is now the most commonly used system for inter-hospital comparison and for identifying any future improvements required for patient safety. There is variation between countries in the CLABSI rates reported by CDC/NHSN and INICC, probably due to inadequate compliance with infection control guidelines, low compliance with hand hygiene and a lack of administrative and financial support [4].

In Saudi Arabia, there are very limited data available on HAIs, especially CLABSI [5, 6]. However, comprehensive strategies to prevent CLABSIs in acute care hospitals have been formulated, e.g. by SHEA and IDSA [7]. Each recommendation is based on scientific evidence and includes administrative and financial guidelines that are measurable and easy to implement. Therefore these recommendations could be used to develop CLABSI prevention models for acute care hospitals in Saudi Arabia and elsewhere.

1.1 BASIC SHEA/IDSA PRACTICES FOR PREVENTION OF CLABSI IN ACUTE CARE HOSPITALS

The SHEA/IDSA basic recommendations on practices to prevent CLABSI in acute care hospitals are summarised in Table 1 [7].

Table 1: *Basic SHEA/IDSA practice recommendations for prevention of CLABSI in acute care hospitals*

Steps	Recommendations
Before insertion of central line	Educate healthcare personnel involved in the insertion, care and maintenance of central venous catheters (CVC) about CLABSI prevention (A-II)
At insertion	1. Avoid using the femoral vein for central venous access in adult patients (A-I) 2. Use maximum sterile barrier precautions during CVC insertion (A-I) 3. Use chlorhexidine-based antiseptic for skin preparation in patients older than 2 months of age (A-I) 4. Use a catheter checklist to ensure adherence to infection prevention practices at the time of CVC insertion (B-II) 5. Perform hand hygiene before catheter insertion or manipulation (B-II) 6. Use all-inclusive catheter cart or kit (B-II)
After insertion	1. For non-tunnelled CVCs in adults and adolescents, change transparent dressing and perform site care with a chlorhexidine-based antiseptic every 5-7 days or more frequently if the dressing is soiled, loose or damp; change gauze dressings every 2 days or more frequently if the dressing is soiled, loose or damp (A-I) 2. Use antimicrobial ointment for haemodialysis catheter insertion sites (A-I) 3. Replace administration sets not used for blood, blood products or lipids at intervals not longer than 96 hours (A-II) 4. Remove non-essential catheters (A-III) 5. Perform surveillance for CLABSI (B-II) 6. Disinfect catheter hubs, needless connectors and injection ports before accessing the catheter (B-II)

The scientifically-based evidence for the SHEA/IDSA basic recommendations, according to criteria grading the strength of the recommendation and quality of evidence, are described in Table 2 [8].

Table 2: *Strength category (A-C) of individual SHEA/IDSA practice recommendations and quality of evidence (grade I-III)*

Category/grade	Definition
Strength of recommendation	
A	Good evidence to support a recommendation
B	Moderate evidence to support a recommendation
C	Poor evidence to support a recommendation
Quality of evidence	
I	Evidence from >1 properly randomized, controlled trial
II	Evidence from >1 well-designed clinical trial, without randomisation; from cohort or case-control analytical studies (preferably from >1 centre); from multiple time series; or from dramatic uncontrolled experiments
III	Evidence from opinions of respected authorities, based on clinical experience, descriptive studies, or reports from expert committees

Note: Adopted from the Canadian Task Force on Periodic Health Examination [8].

1.2 *STAPHYLOCOCCUS AUREUS*

Staphylococci are facultative anaerobic, Gram-positive spherical shaped bacteria that resemble clusters of grapes [9]. Historically, *Staphylococcus aureus* has had the ability to develop resistance to many available antimicrobial agents since 1940s to present. Penicillin was introduced for medical treatment in the 1940s, but *S. aureus* has developed resistance to beta-lactams. In 1960, semi-synthetic penicillin (methicillin) was introduced, but the organism soon developed resistance and the first case of methicillin-resistant *Staphylococcus aureus* (MRSA) was reported in 1961 [10]. The resistance of *S. aureus* to methicillin is caused by the presence of the *mecA* gene, located within a *mec* operon carried by transferrable chromosome staphylococcal cassette chromosome *mec* (SCC*mec*), encoding penicillin-binding protein 2a, which has a low affinity for all beta-lactam antibiotics [11].

Staphylococcus aureus infections are a major cause of community-acquired (CA-) and hospital-acquired (HA-) infections and are associated with significant morbidity and mortality rates. In 1999, NHSN reported that 52.3% of nosocomial infections occurring in ICUs were due to MRSA [12]. HAIs caused by multidrug-resistance have become a serious public health problem, especially in intensive care units (ICUs) due to patients' existing condition, impaired immunity and exposure to

many invasive devices and antibiotic treatments, leading to increasing cost, morbidity and mortality [13-15]. Infections with CA-MRSA in patients with no contact with the hospital environment have been described worldwide [16-19]. Exposure of CA-MRSA strains to the selective pressure of antibiotics used in hospitals has the potential for selection of expanded antibiotic resistance profiles in the pathogen. Consequently, constant re-introduction of MRSA into hospitals from the community reservoir could significantly hamper infection control efforts [18, 20]. CA-MRSA strains are polyclonal, with an epidemiological association between clone type and geographical origin, whereas HA-MRSA infections are caused by a limited number of clones [18, 21, 22].

The rate of MRSA infection ranges from 12% to 49%, with variations in susceptibility pattern profiles, but there are very limited data on the prevalence of different genotypes in Saudi Arabia [23-26]. A systemic review and meta-analysis of the prevalence of MRSA in the Kingdom of Saudi Arabia, based on 26 articles published between 2002 and 2012, revealed variation in the prevalence and antimicrobial susceptibility in patients, but also very limited data on genotyping and virulence gene detection [27]. Panton-valentine leukocidine-positive *Staphylococcus aureus* causes recurring skin and soft tissue infections. Recently, a molecular characterisation study on MRSA infections in Saudi Arabia showed a high diversity clonal complex encoding for PVL and carrying resistance markers in CA-MRSA strains [28]. Vancomycin is the drug of choice for therapy for MRSA infections. However, several countries have reported vancomycin-resistant *S. aureus* (VRSA) [29]. There are two mechanisms of glycopeptide resistance in *S. aureus*. The first is associated with a thickened cell wall [29] and the second type is acquisition of the *vanA* operon, carried by transposon Tn*1546*, resulting in high-level resistance [30, 31].

1.3 MOLECULAR TYPING OF MRSA

Phenotyping systems, such as serotype, biotype, phage type or antibiogram, have been used for many years. Moreover, methods allowing genotyping at molecular level provide the ability to discriminate types of bacteria species and result in high quality, accurate and reproducible data that can be shared globally using software. However, time demands and cost are challenges in application of molecular typing. SCC*mec* typing has been applied in epidemiological studies to distinguish MRSA strains or to define an MRSA clone in combination with the genotype of methicillin-susceptible *S. aureus* (MSSA) strains in which an SCC*mec* element is integrated. SCC*mec* elements consist of: (i) *mec* complex, composed of a *mecA* operon, (ii) *ccr* gene complex, composed of cassette chromosome recombinase (*ccr*) gene(s) and (iii) three regions bordering the *ccr* and *mec* complexes, designated as joining (J) regions. SCC*mec* element typing is defined by a combination of the type of *ccr* gene complex and the class of *mec* gene complex. Eleven SCC*mec* types have been described to date (www.sccmec.org). Recent work by the International Working Group on the Classification of Staphylococcal Cassette Chromosome (SCC) Elements (IWG-SCC) clarified the classification of the major SCC*mec* element types, but there is still a need for clarification regarding naming of the SCC*mec* variants and the accuracy of this typing for epidemiological purposes [11]. Pulsed-field gel electrophoresis (PFGE) is

considered the gold standard among molecular typing methods for a variety of clinically important bacteria, including MRSA [32]. The method has excellent discriminatory power, is inexpensive, has intra-laboratory reproducibility, is a standardised method and is suitable for short-term epidemiological studies. Unfortunately, the method is also time-consuming, labour-intensive and needs experts to interpret the results, especially with bands nearly identical in size. Multi-locus sequence typing (MLST) is a very popular tool for global epidemiology. It is based on sequencing DNA fragments of seven housekeeping gene of *S. aureus* (*arc*C, *aro*E, *glp*F, *gmk*, *pta*, *tpi* and *yqi*L) defined as a sequence type number (ST). The STs are grouped into a clonal complex [3] based on their similarity for six of seven shared alleles, as defined by eBURST (http://eburst.mlst.net) [33]. The tool is excellent to delineate clusters of closely related strains. Limitations of MLST tool are that it is not suitable for characterising differences in strains within outbreak, cost and time consumed. Protein A gene typing is the most widely used method for *S. aureus* genotyping. The polymorphic X region consists of a variable number of 24-bp repeats. These repeats are assigned a numerical code and the *Staphylococcus* protein A gene (*spa*) type is deduced from the order of specific repeats (spaServer.ridom.de). The diversity of the short sequence repeats in a conserved region is suitable for discrimination in outbreak investigation and useful in investigating both the local and global epidemiology of *S. aureus* [32, 34-39]. The complexity and the high cost of polymerase chain reaction (PCR) sequencing analysis are limitations to performing genotyping in developing countries. Recently, high-resolution melting (HRM)-based *spa* typing has been described as an accurate, rapid and cost-effective method for genotyping of MRSA isolates locally [40, 41].

1.4 HIGH-RESOLUTION MELTING

High-resolution melting is the characteristic of a targeted PCR product (amplicon) melting profile resulting from gradually increasing melting temperature (*Tm*) by very small increments in the presence of high-saturating DNA binding dye. As the temperature of the solution is increased, the specific sequence of the amplicon determines the melting behaviour [42]. In general, short amplicons of about 38 to 218 bp in length allow good discrimination to study the pattern of single nucleotide polymorphisms (SNPs) [43, 44]. Analysis based on HRM has been used in different clinical laboratory applications such as detection of the alpha-thalassemia-1 Southeast Asian allele [45], mutation detection in fibroblast growth factor receptor 3 gene [46], screening HLA identity between individuals as a possible alternative to current molecular and serological HLA typing [47], influenza A subtypes [48], herpes simplex virus (HSV) typing [49], identification of many different bacterial isolates [50], differentiation of Mycobacteria species [51] and *S. aureus* genotyping [40, 41]. The LightCycler® Nano system for HRM analysis is fast, highly sensitive and produces reproducible results for qualitative and quantitative detection of nucleic acids, melting curve analysis, SNP genotyping and mutation scanning. The software has no licence restrictions (www.lifescience.roche.com/shop/products/**lightcycler-nano**-instrument). A study by Chen *et al.* [41] demonstrated the robustness and reliability of HRM-based real-time PCR for MRSA spa typing using

the LightCycler® Nano system. Thus HRM *spa* typing can be applied in clinical diagnostic laboratories for epidemiological analysis and infection control of MRSA [41]. The Rotor-Gene 6000 system enables real-time amplification for HRM analysis, with the rotary design giving great advantages in terms of thermal and equilibration time uniformity. To ensure accuracy, the Rotor-Gene 6000 system uses an Optical Temperature Verification (OTV™) rotor that automates verification testing. Each test takes only minutes and can be repeated at any time and as often as required. The proprietary software has the ability to calculate the efficiency of individual amplification reactions and also has biostatistics and graphing capabilities. (www.biolabo.com/**Real-Time**-PCR-Detection-**System-Rotor-Gene-6000**). Stephens *et al.* successfully discriminated 22 different MRSA *spa* types using the Rotor-Gene 6000 system [40]. HRM-based *spa* typing has been described as a rapid and cost-effective method for genotyping locally predominant isolates [40, 41].

1.5 SHIGELLOSIS

Shigellosis is an infectious diseases caused by various species of *Shigella*. The organism was discovered by Japanese microbiologist, Shiga, and later the genus given under his name. Shigellae are Gram-negative, rod-shaped, non-motile, and facultative anaerobic. They are highly infectious with incubation period of 12 hours up to 7 days. There are four Shigella species; *Shigella dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*, which are classified into four sero-group A, B, C and D respectively, based on biomedical and serological differences. Clinical feature of the disease is characterized by diarrhea contain blood and mucus, vomiting, fever and abdominal cramps [52, 53]. The annual number of Shigella episodes throughout the world was estimated to be 164.7 million, of which 163.2 million were in developing countries and 1.5 million in industrialized countries. The incidence rate varies according to age, geographical distribution, and local human development index (HDI). In developing countries, *S. flexneri* is the most commonly detected strain followed by *S. sonnei* strains. *S. dysenteriae* and *S. boydii* occur equally frequently. In contrast, *S. sonnei* is the most common serogroup found in industrialized countries followed by *S. flexneri*, *S. boydii* and *S. dysenteriae*. The variation incidence refers to different health infrastructure, surveillance system and laboratory diagnosis facilities between developing and industrial countries [54]. Sulfonamides, tetracycline, ampicillin, trimethoprim-sulfamethoxazole, nalidixic acid, and pivmecillinam have been used as first-line antimicrobial drugs in many parts of the world [55]. However, Shigella has demonstrated extraordinary prowess in acquiring plasmid-encoded resistance to many antimicrobial agents. There are also geographic differences in the resistance rates suggesting the occurrence and spread of particular antimicrobial-resistant clones [54].

1.6 *SHIGELLA SONNEI*

Shigella sonnei has been predominantly responsible for dysentery in developed countries, but is now emerging as a problem in the developing world. In the United States [56], *Shigella* infections have not declined appreciably over the past 10 years and *Shigella sonnei* is responsible for the largest

percentage of infections (75%), followed by *S. flexneri* (12%), *S. boydii* (0.8%) and *S. dysenteriae* (0.3%). The incidence rate of infection with *S. sonnei* decreased from 2008 to 2011, but increased in 2012 [56]. Analysis following a recent outbreak in the United States demonstrated that the organism concerned displayed high minimum inhibitory concentrations (MICs) of azithromycin. Isolates were found to harbour macrolide resistance gene, *mphA*, located on a plasmid DNA [57].

1.7 MOLECULAR TYPING OF *S. SONNEI*

Shigella sonnei has only one serotype and is genetically homogeneous, but many genotyping methods have shown variations in the genome DNA and can distinguish between individual epidemic strains. Nevertheless, these methods may not be suitable for studying the variation and evolution of isolates within clone collected over long periods of time. One study found clear evidence of temporal variation over a 41-year period in frequency of a particular restriction site, which could be due to either periodic selection or random genetic drift within a clone of *S. sonnei* [58].

To establish an accurate population, Holt *et al.* [59] sequenced the whole genome of 132 *S. sonnei* isolates collected globally between 1943 and 2008. They detected 10,111 chromosomal single nucleotide polymorphisms (SNPs) randomly distributed around the chromosome. Phylogenetic study methods showed a strong correlation between root-to-tip branch length and the known date of isolation, indicating rapid, clock-like evolution over 500 years. There is some rate variation between four lineages and, in addition, there are several geographically associated, multi-drug resistance clones that belong to specific lineage III [59]. Recently, Vartul S. *et al.* found that 97 SNPs were phylogenetically informative and can reliably discriminate the known *S. sonnei* lineages, a finding confirmed by sequencing analysis. Furthermore, six SNPs can define lineages/sub-lineages due to linkage association between 97 SNPs [60]. Several molecular typing methods, including MLST and PFGE, have been applied to *S. sonnei* in evolutionary and epidemiological studies. These methods are more labour-intensive, expensive, require special expertise and the results are difficult to interpret. An accurate, rapid and cost-effective typing scheme is urgently needed for active surveillance and epidemiological investigations. HRM typing is a highly sensitive, rapid and cost-effective option for such purposes. HRM data analysis does not require expert training and the method can be easily implemented in reference laboratories across the globe. It has been applied to *Salmonella* spp. studies [61]. HRM studies have also confirmed that the current global burden involves several geographically associated and multidrug-resistant clones that belong to a single lineage [59, 60].

2 AIM OF THE THESIS

In 2011, the annual risk assessment of King Abdul Aziz Specialist hospital, Taif, Saudi Arabia, reported a high incidence of CLABSI and found that the characteristic susceptibility pattern of CA-MRSA infections in the hospital was different from that at other locations (data not published). Based on these findings at the hospital, the aim of this thesis was to develop plans to reduce CLABSI, based on SHEA/IDSA practice guidelines, and to determine the epidemiological distribution of MRSA infections in Taif city. It was decided to use HRM as the genotyping method in laboratory diagnostics.

The specific aims of the studies described in Papers I-IV were to:

- Reduce central line-associated bloodstream infections in trauma intensive care units using basic SHEA/IDSA practice guidelines (Paper I)
- Characterise MRSA infections in Taif city, Saudi Arabia (Paper II).
- Evaluate the applicability of HRM MRSA *spa* typing (Paper III)
- Introduce a new tool for HRM *Shigella sonnei* genotyping (Paper IV).

3 METHODOLOGICAL CONSIDERATIONS

3.1 POPULATION (PAPER I)

The work described in Paper I study was conducted from January 2011-December 2012 at the 23-bed trauma ICU at King Abdul Aziz Specialist Hospital in Taif (KAASH-Taif).

3.2 CRITERIA OF CLABSI (PAPER I)

Criteria of CLABI were defined according to NHSN guidelines [62], whereby: Laboratory-confirmed (LC) CLABSI is a primary bloodstream infection (BSI) in a patient who had a central line in place at the time of, or within 48 hours before, onset of BSI. LC-BSI must meet one of the following three criteria:

1. Patient has a recognised pathogen cultured from one or more blood cultures and an organism cultured from blood in, not related to, an infection at another site.
2. Patient has at least one of the following signs or symptoms:
   - fever (>38 ^{o}C), chills, hypotension
   - signs, symptoms and positive laboratory results are not related to an infection at another site
   - common skin contamination is cultured from two or more blood cultures drawn on separate occasions.
3. Patient <1 year of age has at least one of the following signs or symptoms:
   - fever (>38 ^{o}C rectal), hypothermia (37 ^{o}C rectal), apnoea or bradycardia
   - signs, symptoms and positive laboratory results are not related to an infection at another site
   - common skin contamination is cultured from two or more blood cultures drawn on separate occasions.

3.3 HAND HYGIENE OBSERVATION (PAPER I)

Hand hygiene observation (HHO) was conducted using the WHO “My five moments” for hand hygiene recommendations. Calculations and statistical analyses were performed according to WHO recommendations [63, 64].

3.4 BACTERIAL ISOLATES AND IDENTIFICATION

For Paper I, bacterial identification and determination of susceptibility to a range of antimicrobials were carried out according to Clinical Laboratory Standards Institute [65] guidelines using the Vitek 2system (BioMerieux, USA) [65, 66].

For Paper II, 28 clinical MRSA isolates were collected randomly from three referral hospitals in Taif between January and August 2011. Methicillin-resistant phenotype was confirmed according to British Society for Antimicrobial Chemotherapy [67] standards using the Vitek2 system (BioMerieux,

USA). An isolate is considered methicillin resistant when the minimum inhibitory concentration (MIC) breakpoint of oxacillin is >2 mg/L and of cefoxitin >4 mg/L [67].

For Paper III, 50 clinical MRSA isolates collected between 2005 and 2012 were selected randomly from Scotland (n=22), Brazil (n=13), Sudan (n=3) and Saudi Arabia (n=12). All *S. aureus* isolates were identified as described previously; white colonies on blood agar plate, cocci bacterial shape and positive to Gram stain, catalase and coagulase [9]. Methicillin-resistant phenotype was determined in accordance with BSAC standards using the Vitek 2system (BioMerieux, USA). An isolate was considered MRSA when the MIC breakpoint of oxacillin was >2 mg/L and of cefoxitin >4 mg/L [67].

For Paper IV, 10 *Shigella sonnei* DNA samples (two for each lineage/sublineage) were provided by Institut Pasteur, France. However, lineage IV strain was not included in the study.

3.5 ANTIMICROBIAL SUSCEPTIBILITY TESTING (PAPER II)

The MIC of 17 antimicrobial agents, namely cefoxitin, chloramphenicol, ciprofloxacin, clindamycin, daptomycin, erythromycin, fusidic acid, gentamicin, linezolid, mupirocin, nitrofurantoin, oxacillin, penicillin, rifampicin, tetracycline, teicoplanin and vancomycin, was determined according to BSAC standards using the Vitek2 system (BioMerieux, USA).

3.6 DNA EXTRACTION (PAPER II AND III)

Genomic DNA was isolated from a 2-mL overnight culture with the DNeasy tissue kit (QIAGEN, Hilden, Germany), using lysostaphin (100 mg/L; Sigma, Taufkirchen, Germany).

3.7 DETECTION OF PVL AND MECA GENES (PAPER II)

A PCR was carried out for *PVL* and mecA gene amplification individually in two runs using a thermo cycler (Eppendorf, Hamburg, Germany) as described previously with modifications [68]. In brief, 20 µL of final reaction mixture of each run containing 10 µL of 1X Taq master-mix reaction, 1 µL of each primer (100µM) (*luk*-PV-F: 5`-ATCATTAGGTAAAATGTCTGGACATGATCCA-3`, *luk*-PV-R: 5`- GCATCAAGTGTATTGGATAGCAAAAGC-3`, and mecA-F: 5`-GTAGAAATGACTGAACGTCCGATAA-3`, mecA-R: 5`-CCAATTCCACATTGTTTCGGTCTAA-3`) and 2 µL of the DNA (20 ng) template were used. PCR conditions were as follows: initial denaturation at 95 °C for 10 min, followed by 25 cycles of denaturation at 95 °C for 1 min, annealing at 74 °C for 1 min and extension at 72 °C for 1 min and a final extension step at 72 °C for 10 min. MRSA ATCC 35591 and DNase/RNase-free distilled water were included as positive and negative controls, respectively. For visualisation of the product, 10 µL of PCR amplicons were mixed with 1 µL of EzVision One loading dye (Amresco, Solon, OH, USA) and loaded into a 1.5% (wt/vol) agarose gel (Agarose I^{TM}). Electrophoresis was carried out in 1X TAE buffer at 80 v for 50 minutes. A molecular weight marker 100-bp ladder (Promega, Madison, WI,

USA) was included on each gel. Bands were visualised using an Alpha Innotech UV imager (FluorChem™).

3.8 HA-MRSA AND CA-MRSA INFECTIONS CRITERIA (PAPER II)

Identification of HA-MRSA and CA-MRSA infections was based on hospitalisation history and site of infection according to CDC/HNSN criteria [62]. An isolate was defined as HA-MRSA if the MRSA-positive specimen was obtained 2 days after hospital admission and met CDC site infection criteria. An isolate was defined as CA-MRSA if the MRSA-positive specimen was obtained within 48 hours of admission, with no history of MRSA infection within a year as a risk of acquired MRSA.

3.9 *SPA* AND MLST TYPING (PAPERS II AND III)

The polymorphic regions of the *spa* gene and seven housekeeping genes (*arc*, *aroE*, *glp*, *gmk*, *pta*, *tpi* and *yqiL*) were amplified by PCR using the primers listed in Table 3, as described previously [34, 69]. All sequencing reactions were carried out using the ABI PRISM BigDye Terminator cycle sequencing ready reaction kit (Applied Biosystems, Foster City, California, USA). The sequence data were analysed in BioNumerics v.5.1 (Applied Maths).

Table 3: *List of primers used for genotyping*

Gene	Forward 5`-3`	Reverse 5`-3`
Spa	AGACGATCCTTCGGTGAGC	GCTTTTGCAATGTCATTTACTG
Arc	TTGATTCACCAGCGCGTATTGTC	AGGTATCTGCTTCAATCAGCG
aroE	ATCGGAAATCCTATTTCACATTC	GGTGTTGTATTAATAACGATATC
Glp	CTAGGAACTGCAATCTTAATCC	TGGTAAAATCGCATGTCCAATTC
Gmk	ATCGTTTTATCGGGACCATC	TCATTAACTACAACGTAATCGTA
Pta	GTTAAAATCGTATTACCTGAAGG	GACCCTTTTGTTGAAAAGCTTAA
Tpi	TCGTTCATTCTGAACGTCGTGAA	TTTGCACCTTCTAACAATTGTAC
Yqi	CAGCATACAGGACACCTATTGGC	CGTTGAGGAATCGATACTGGAAC

3.10 HRM ANALYSIS FOR *SPA* TYPING (PAPER III)

The polymorphic X region of the *spa* gene was amplified in a Rotor-Gene 6000 instrument (Qiagen) using the SensiMix™ HRM kit (Bioline) as previously described [70]. In brief, a 20 µL PCR reaction was set up, containing 0.8 µL Eva-Green, 10 µL SensiMix™, 1 µL of each primer (100µM; 1095 forward 5`-AGACGATCCTTCGGTGAGC-3` and 1517 reverse 5`-GCTTTTGCAATGTCATTTACTG-3`) and 20 ng of the template DNA and programmed as follows: a hold at 95 °C for 10 min followed by 35 cycles of 95 °C for 20 s, 56 °C for 20 s and 72 °C for 22 s. The HRM of the amplicons was performed between 70 and 95 °C with a stepwise increase of 0.05 °C.

The melting temperatures (*Tm*) were determined by the negative derivative of decreased fluorescence over increased temperature (*df/dt*), using the proprietary software (version 1.7.34). The shapes of the melting curves were viewed with the same software.

3.11 MULTIPLEX HRM ANALYSIS FOR *SHIGELLA SONNEI* GENOTYPING (PAPER IV)

Primers were designed to amplify single nucleotide polymorphisms (SNPs) within *kduD*, *deoA* and *emrA* for typing the three main lineages in a first set run and to amplify SNPs within *fdX* and *menF* for typing sub-lineages in a second set run (Table 4). For the first run, a 20 µL multiplex real-time PCR mixture containing 10 µL master mix (SensiMix with EvaGreen dye (Bioline)), 20 ng of template DNA and 2.5 µL of each primer of *kduD*, 1 µL of each primer of *deoA* and 1 µL of each primer of *emrA* was used. A similar reaction mix was used for each run, except that the primers used were 2 µL of each primer of *fdX* and 1 µL of each primer of *menF*. The multiplex PCR cycling was programmed as follows: the mixture was held at 95 °C for 10 min and then subjected to 35 cycles of 95 °C for 20 s, 60 °C for 20 s and 72 °C for 22 s. The HRM was performed between 80 and 90 °C with a stepwise increase of 0.05 °C. The *Tm* was determined by the negative derivative of decreased fluorescence over increased temperature (*df/dt*), using the proprietary software (version 1.7.34).

3.12 REPRODUCIBLE HRM GENOTYPING OF *SHIGELLA SONNEI* (PAPER IV)

To assess the reproducibility, 10 samples of various lineages were randomly run blind under the same conditions.

Table 4: *Primers used for multiplex-HRM typing of Shigella sonnei*

Target of primers	Direction	Sequence
kduD	Forward	5´-CGACGGCGAAACACTTTATC-3`
	Reverse	5´-CGCGTATAAGAAGGCACACG-3`
deoA	Forward	5´-GGAGATGCTTATCTCCGGCAAA-3`
	Reverse	5´-AGTCGGTTGGGCCTTTT-3`
emrA	Forward	5`-TGCCACCGAAGTACGTAACG-3`
	Reverse	5`-CATCCACCCACATATTGGTG-3`
fdX	Forward	5`-CAAAGCCTGGGACTGGA-3`
	Reverse	5`-CATGGTTGATAGTGTAACGC-3`
menF	Forward	5`- TATTCTCGCGCTGGTTTTTA-3`
	Reverse	5`-GCTTTTCTTGGCTCTTCACC-3

4 RESULTS

4.1 INSTITUTIONAL RISK ASSESSMENT (PAPER I)

Observations revealed that there were three potential risk factors did not meet SHEA/IDSA basic recommendations: training of healthcare workers, removal of non-essential catheters and use of a catheter cart. All three risk factors were addressed.

4.2 HAND HYGIENE OBSERVATION (PAPER I)

The hand hygiene compliance rate ranged from 39% to 70% and the alcohol hand rub consumption rate was maintained at 43% during the study period.

4.3 INCIDENCE RATE AND RATIO OF CLABSI P INTERVENTIONS (PAPER I)

In 2011, there were 14 cases of CLABSI and the incidence rate was 3.87 per 1000 central line days with utilisation ratio of 0.53. In 2012, following implementation of basic SEHA/ISDA practice recommendations on hospital hygiene, the number of CLABSI cases was six and the incidence rate was 1.5 per 1000 central line days, with a utilisation ratio of 0.60. A summary of CLABSI cases, rate and ratio benchmarking to NHSN hospitals in 2011 and 2012 is presented in Table 5.

Table 5: *Incidence rate and ratio of CLABSI reported during the study period (2011-2012) at the study hospital compared with NHSN hospitals (DA Module 2010)*

Year	Number of CLABSIs	Device days	Patient days	Rate/1000 device days	Comparison with NHSN percentiles	Ratio	Comparison with NHSN percentiles
2011	14	3.616	6.835	3.87	75 – 90	0.53	25 – 50
2012	6	4.134	6.864	1.5	50	0.60	50 – 75

4.4 IDENTIFICATION OF MICROORGANISMS CAUSING CLABSI (PAPER I)

Microorganisms identified as causing CLABSI are listed and their incidence is summarised in Table 6. It was found that CLABSIs were caused predominantly by multidrug-resistant bacteria, namely; *Klesiella pneumonia*, *Enterococcus faecalis* and *Acinetobacter baumannii*.

Table 6: *Type and incidence of microorganisms causing CLABSI during the study period*

Microorganisms	Number of isolates per year	
	2011	2012
Klebsiella pneumonia	3	3
Enterococcus faecalis	2	2
Acinetobacter baumannii	2	1
Enterobacter cloacae	2	0
Escherichia coli	1	0
Pseudomonas aeruginosa	1	0
Methicillin resistant Staphylococcus aureus	1	0
Proteus mirabilis	1	0
Burkholderia spp	1	0
Total	14	6

4.5 SUSCEPTIBILITY PATTERN OF MRSA ISOLATES (PAPER II)

All MRSA isolates proved to be susceptible to chloramphenicol, daptomycin, nitrofurantoin, teicoplanin, rifampicin and vancomycin. However, resistance rates varied as follows: fusidic acid (46%), tetracycline (39%), ciprofloxacin (36%), trimethoprim (28%), gentamicin (25%), clindamycin (21%), erythromycin (14%) and mupirocin (7%). Three strains demonstrated resistance to eight antimicrobial agents (ciprofloxacin, clindamycin, erythromycin, fusidic acid, gentamicin, tetracycline, trimethoprim and mupirocin).

4.6 *SPA* AND MLS GENOTYPING OF MRSA ISOLATES (PAPER II)

Twelve *spa* types were identified, including *t*4573 (21%) followed by *t*304 (18%) and *t*044, *t*267 and *t*363 (10% of each). Eight multi locus sequence types (MLSTs) were identified, with the majority being sequence type (ST) ST-22 (32%), followed by ST-80 and ST-97 (14%), ST-2882 (11%), ST-241 (7%) and ST-239, ST-5 and ST-6 (3%). The *t*4573/ST-22 genotype was most prevalent in the hospital (46%).

4.7 PVL AND MECA GENES DETECTION (PAPER II)

All isolates tested positive for *mec*A gene. Thirteen MRSA isolates (46%) tested positive for *PVL* gene; 54% related to HA-MRSA infection and 46% to CA-MRSA infection. All *t*4573/ST22 strains were resistant to ciprofloxacin, harboured *PVL* gene and were related to surgical site infection, skin soft tissue infections and pneumonia (Table 7).

Table 7: *Genotypes and susceptibility pattern of MRSA infections observed at KAASH-Taif, Saudi Arabia, 2012*

Spa-type (N)	MLST (N)	*PVL* (N)	Resistant to
*t*304 (5)	2882 (3)	-ve (3)	
	6 (2)	-ve (1)/ +ve (1)	
*t*311 (1)	5 (1)	-ve (1)	Cli
*t*2770 (2)	97 (5)	-ve (2) / +ve (1)	Fa
*t*267 (3)		-ve (2)	Fa, Te, Gan
*t*044 (3)	80 (4)	+ve (5)	Fa, Te
t131 (1)			
*t*037 (1)	239 (1)		
*t*4573 (6)	22 (9)	-ve (1)/ +ve (5)	Cip
			Cip, Tr
			Cip, Cli, Tr, Gen, Ery
*t*748 (1)		+ve (1)	Cip, Tr
*t*5716 (1)		-ve (2)	Cli, Tr
t223 (1)			Tr, Te
*t*363 (3)	241 (3)	-ve (3)	Cli, Fa, Cip, Tr, Te, Gen, Ery, Mu
			Cli, Fa, Cip, Tr, Te, Gen, Ery, Van

Cli: clindamycin, Fa: fucidic acid, Cip: ciprofloxacin, Tr: trimethobrium, Te: tetracycline, Gen: gentamicin, Ery: erythromycin, Mu: muperocin

4.8 HRM *SPA* TYPES IDENTIFICATION (PAPER III)

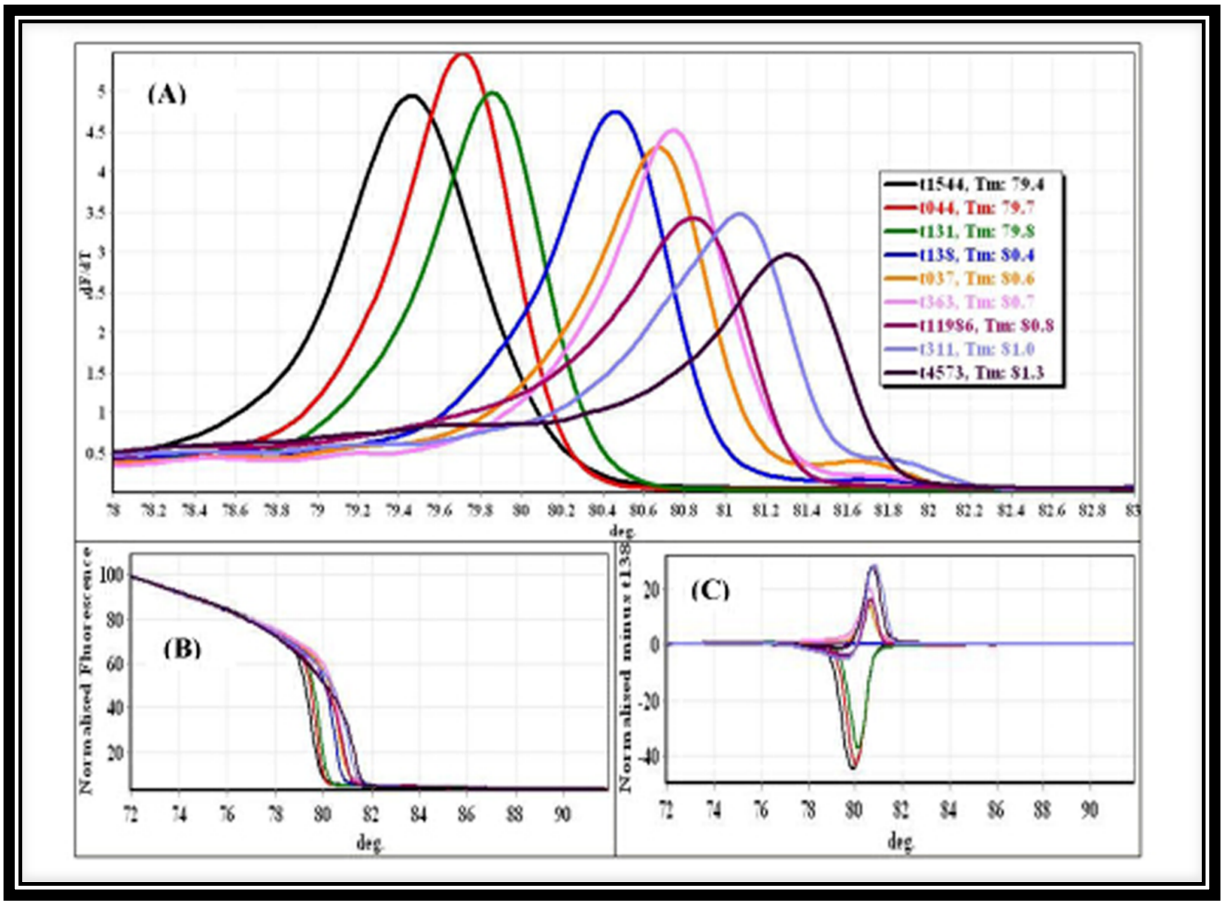
Nineteen *spa*-types were identified among 50 clinical MRSA isolates. *Spa* type *t*037 was the prevalent type among Brazilian isolates (12/13) and Sudanese isolates (3/3). Eight *spa* types were observed among 22 isolates from Scotland and nine *spa* types among 12 isolates from Saudi Arabia.

Fifteen out of the 19 *spa* types detected each had a distinct melting temperature (*Tm*) that unambiguously assigned 44 isolates (Table 8, Figure 1). Both *t*008 and *t*2770, as well as *t*311 and *t*021 *spa* types, shared the same *Tm* (80.3 and 81.0 °C, respectively) (Figure 2)

Table 8: HRM and spa *sequences of MRSA isolates and frequencies in different countries*

HRMT	*Tm*	*Spa*-type	Repeat of *spa* type	Size-	CG %	Country (N. of *spa* types/total)
1	79.4	*t*1544	07-22-34	72	44.4	Saudi Arabia (2/12)
2	79.5	*t*344	09-02-16-13-34	12	50	Scotland (5/22)
3	79.7	*t*044	07-23-12-34-34-33-34	16	41.7	Saudi Arabia (1/12)
4	79.8	*t*131	07-23-12-34-33-34	14	42.3	Saudi Arabia (1/12)
5	80.2	*t*304	11-10-21-17-34-24-34-22-25	21	43.5	Saudi Arabia (1/12)
6	**80.3***	***t*2770**	**07-23-12-12-21-17-34-34-33-34**	**24**	**42.5**	**Saudi Arabia (2/12)**
		***t*008**	**11-19-12-21-17-34-24-34-22-25**	**24**	**43.7**	**Scotland (1/22)**
7	80.4	*t*138	08-16-02-25-17-24	14	45.1	Brazil (1/13)
8	80.6	*t*037	15-12-16-02-25-17-24	16	45.2	Brazil (12/13) and
9	80.7	*t*363	15-16-02-25-17-24	14	45.8	Saudi Arabia (1/12)
10	80.8	*t*1198	04-44-33-31-31-12-34-16-12-25-22-34	28	43.5	Saudi Arabia (1/12)
11	80.9	*t*018	15-12-16-02-16-02-25-17-24-24-24	26	44.8	Scotland (6/19)
12	**81.0***	***t*311**	**26-23-17-34-20-17-12-17-16**	**21**	**44.9**	**Saudi Arabia (1/12)**
		***t*021**	**15-12-16-02-16-02-25-17-24**	**21**	**44.9**	**Scotland (2/22)**
13	81.2	*t*002	26-23-17-34-17-20-17-12-17-16	24	45.4	Scotland (2/22)
14	81.3	*t*4573	07-23-13-23-31-05-17-25-16-28	24	45.0	Saudi Arabia (2/12)
15	81.4	*t*020	26-23-31-29-17-31-29-17-25-17-25-16-28	31	44.5	Scotland (1/22)
16	81.8	*t*4291	26-23-23-13-23-31-29-17-31-29-17-25-16-25-16-28	36	43.8	Scotland (1/22)
17	81.9	*t*032	26-23-23-13-23-31-29-17-31-29-17-25-17-25-16-28	38	45.0	Scotland (4/22)

**spa* types in bold share the same melting temperature (*Tm*) with another *spa* type.


(A)
dF/dT
deg.
t1544, Tm: 79.4
t044, Tm: 79.7
t131, Tm: 79.8
t138, Tm: 80.4
t037, Tm: 80.6
t363, Tm: 80.7
t11986, Tm: 80.8
t311, Tm: 81.0
t4573, Tm: 81.3
(B)
Normalised Fluorescence
deg.
(C)
Normalised minus t138
deg.

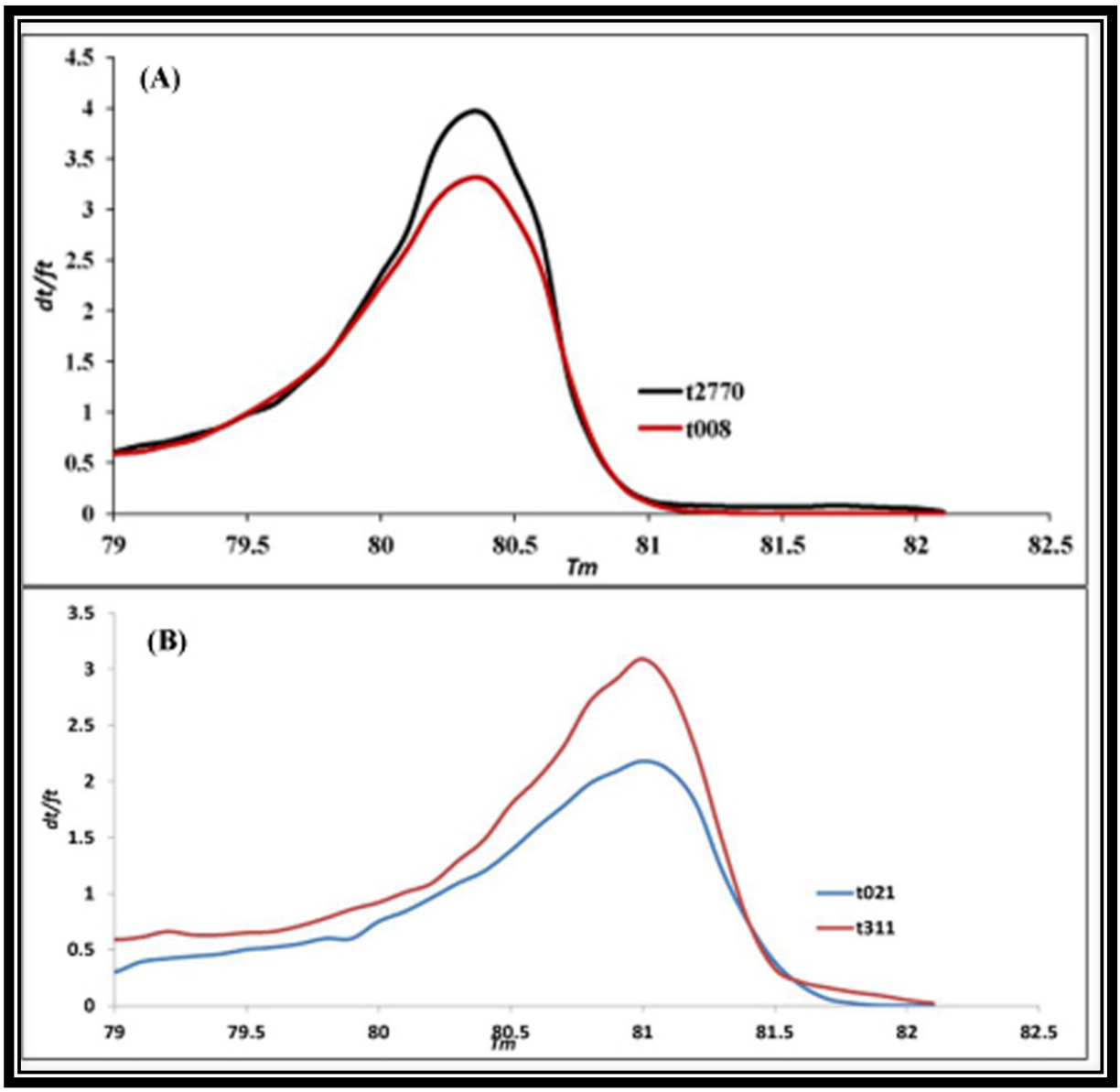


Figure 2: Melting curve shapes allowing assignment of *spa* types that shared the same melting temperature (*Tm*). (A) Characteristics of melting curve shapes for *t*2770 and *t*008, which had identical *Tm* = 80.3 °C. (B) Characteristics of melting curve shapes for f t021 and t311, which had identical *Tm* = 81.0 °C.

4.9 HRM GENOTYPING OF *SHIGELLA SONNEI* (PAPER IV)

The first set run separated lineages I, II and III with distinctive melting curves and the *Tm* of each allele was at least a half degree away from that of other alleles (Figure 3A). The second set run distinguished the sublineages IIIa, IIIb and IIIc with distinctive melting curves (Figure 3B). The *Tm* references for all alleles are summarised in Table 9.

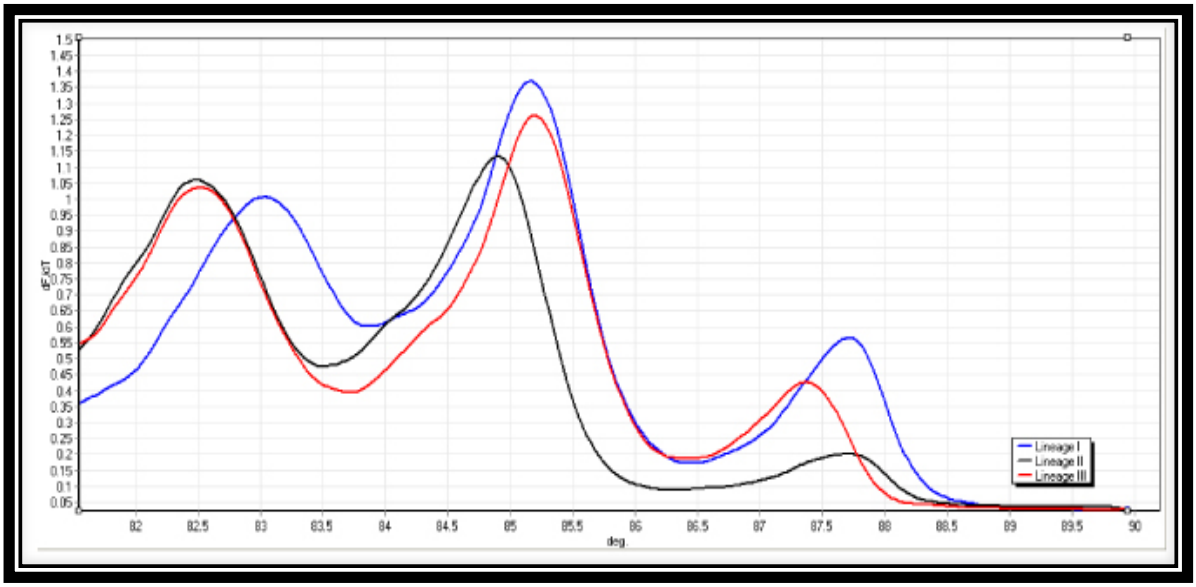


Figure 3A: Multiplex-HRM curve of three single nucleotide polymorphisms (SNPs) in *kduD*, *deoA* and *emrA*, respectively (from left to right), which separate *Shigella sonnei* lineages I, II and III.

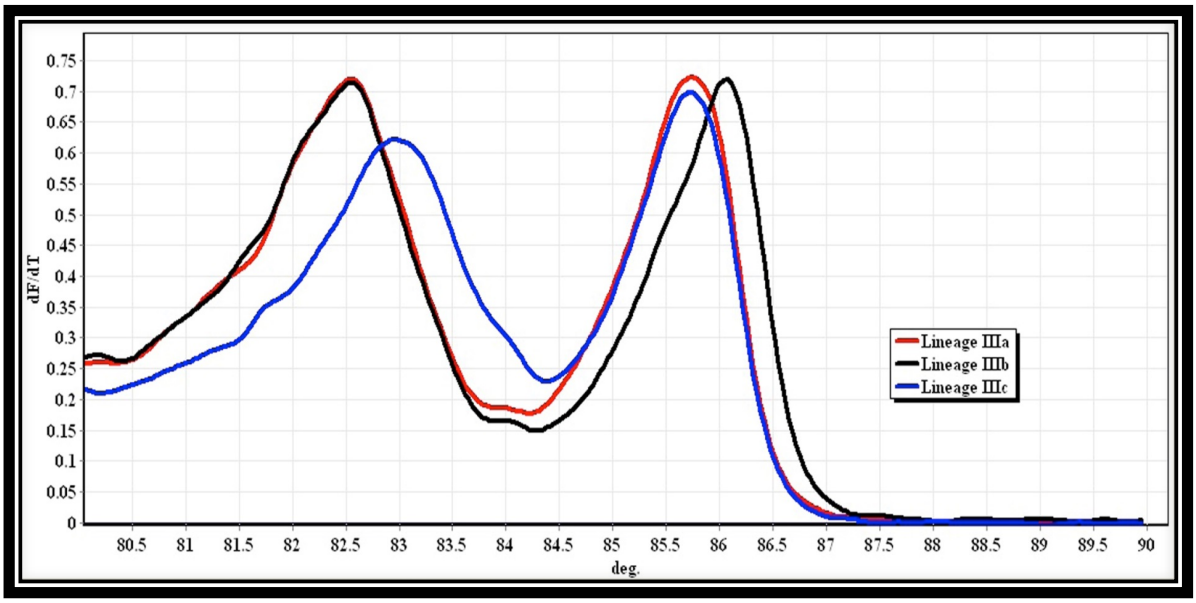


Figure 3B: Multiplex-HRM curve of two single nucleotide polymorphisms (SNPs) in *fdX* (left) and *menF* (right), which separate lineages IIIa, IIIb and IIIc.

Table 9: *Summary of melting temperature (Tm) for each of the five single nucleotide polymorphisms (SNPs) used for multiplex-HRM analysis*

Lineages	*Tm* (° C) (+/- 0.05) for:				
	kduD	*deoA*	*emrA*	*fdX*	*menF*
Mail lineages					
I	83.0	85.1	87.7		
II	82.5	84.8	87.7		
III	82.5	85.2	877.3		
Sub lineages					
IIIa				82.5	85.7
IIIb				82.5	86.0
IIIc				82.9	85.7

4.10 REPRODUCIBLE HRM GENOTYPING OF *SHIGELLA SONNEI* (PAPER IV)

A randomly selected 10 samples of different lineages revealed similar results, with +/-0.05 differences in *Tm*.

5 DISCUSSION

CLABSI is an important cause of increased morbidity, mortality, increased duration of hospital stay and excess healthcare costs in ICUs [7]. In Saudi Arabia, very limited data on CLABSI in adult ICUs are available. The CLABSI incidence rate to percentile NHSN benchmarking detected in this thesis in 2011 indicated a need to improve patient care practice in the trauma ICU in KAASH-Taif hospital. Therefore, it was decided to introduce a strategic plan to reduce CLABSI and deliver high quality performance, as well as patient safety.

Basic SEHA/ISDA practice recommendations were chosen for this purpose due to their concise format and applicability in the hospital setting and their inclusion of measurable elements. There was a significant reduction in CLABSI after intervention implementing the SEHA/ISDA practice recommendations. However, the difference in the annual rate of incidence of CLABSI before and after resolving risk factors was not statistically significant due to low number of cases in the study period. Nevertheless, the incidence rates were maintained within the pooled mean in 2012 (Figure 4).

It is recommended that device-associated infection rates and device utilisation ratios be examined together, so that preventive measures may be appropriately targeted [71]. The 90th percentile NHSN benchmarking of CLABSI incidence rate with the 50th percentile NHSN benchmarking of utilisation ratio indicated that prevention measures should be focused on before and during insertion of a central line. After the intervention period, CLABSI incidence declined to the 50th percentile of NHSN benchmarking and the utilisation ratio to a higher (75th) percentile of NHSN benchmarking, indicating staff training, catheter cart preparation and removing unnecessary central lines as important prevention measures.

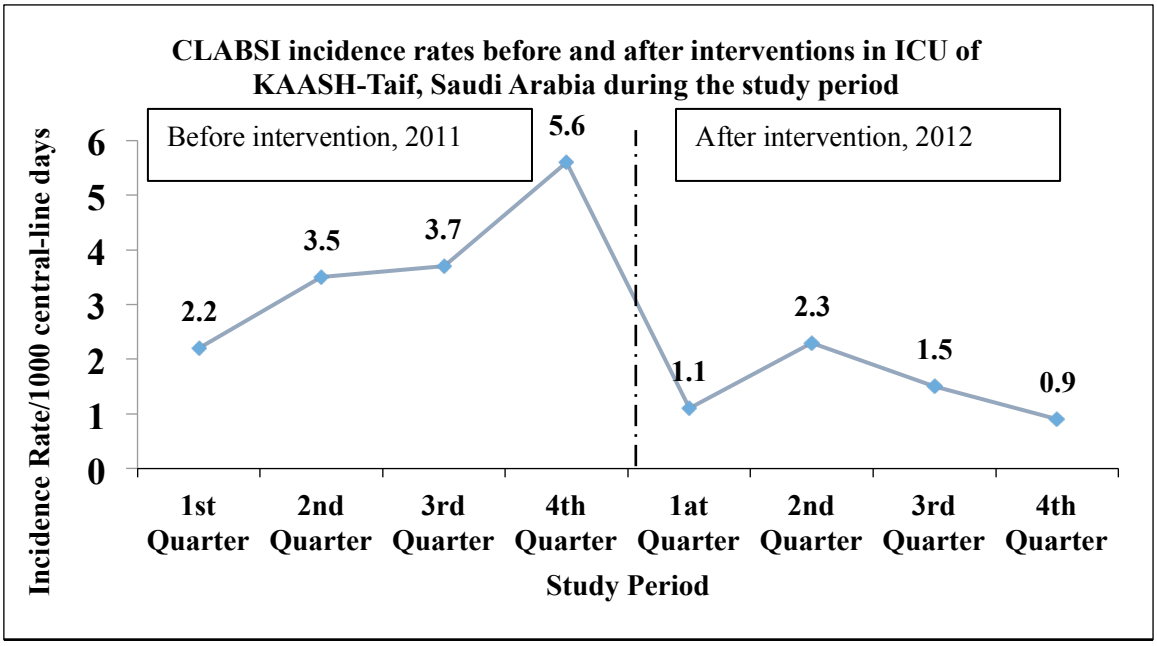


Figure 4: Incidence of CLABSI reported before and after implementation of SEHA/ISDA practice recommendations.

Lack of familiarity with practice guidelines by healthcare workers has been identified as a main barrier to the proper implementation of these guidelines. Alfonso *et al.* observed a significant reduction (35%) in CLABSI in ICUs after training interventions and noted that a programme of continual training is the first step toward achieving compliance with guidelines [72]. The use of a central line monitoring protocol in the study hospital ensured prompt removal of non-essential central line catheters. The method was easily reproducible, with positive feedback from healthcare workers. The introduction of a catheter cart and kit made it easy for staff to access and adhere to preventive measures during insertion of a central line catheter. Regular meetings with the CLABSI prevention team and ICU department were held, with dissemination of feedback to all stakeholders.

However, despite regular reminders about the importance of hand hygiene, the compliance rates were variable (39-70%, average rate 56%) and with a low alcohol consumption rate of 43% (p=0.15). This is perhaps due to the fact that the ICU is a very busy care area. The significant reduction in CLABSI in the trauma ICU studied indicates the positive impact of addressing these three risk factors. However, it is clear that regular training programmes, particularly with respect to hand hygiene compliance, should be held regularly to maintain high performance in patient care services.

The incidence rates and susceptibility patterns to antimicrobial agents are variable across geographical regions, due to many factors such as surveillance methods, economic status and use of antimicrobials. There is wide variation in the antimicrobial susceptibility pattern in Saudi Arabia. Rifampicin resistance has previously been observed in Al Khubar (76%), Qassem (60%), Taif and Riyadh (33%), Aseer (11%(and Makkah (6%), but not in Najran city [73-79]. Chloramphenicol resistance has been recorded in Taif (30%) and Asser (1.5%) [74, 75]. Only one case of daptomycin resistance has been reported in Saudi Arabia, in Najran (9.4%) [77]. This variation can be assumed to be a reflection of differences in study design and surveillance method.

Multidrug-resistant *S. aureus* has been reported in many hospitals world-wide but, to the best of my knowledge, this thesis is the first study to report multi-drug resistant MRSA with resistance to ciprofloxacin, clindamycin, erythromycin, fusidic acid, gentamicin, mupirocin, tetracycline and trimethoprim in Saudi Arabia.

A diversity of *spa* and MLST types were identified in Taif, with *t*4573/ST-22 being the most prevalent in the hospital (21%). Similar diversity has been reported in Riyadh [79, 80].

Interestingly, although *t*4573, harbouring *PVL* gene and resistant to ciprofloxacin, is common in Taif city (n=6, 21%), the strain has a very rare distribution worldwide and is not well characterised. One isolate has been reported in Saudi Arabia (Riyadh) [79], one in New Zealand [81] and two in Sweden (www.spaserver.ridom.de). One isolate of *t*037 was detected in Taif, but *t*037 is common in Riyadh (35%) and has a wide global distribution. This variation probably reflects the low sample collection in the present study.

Clonal complex (CC) 22 is the most common MLST type (n = 9, 32%) [28]. *PVL*-positive MRSA strains are associated with complicated infections; especially skin infections and necrotising pneumonia.

High prevalence of the gene encoding *PVL* was noted in Taif city (46%) and tended to be more associated with HA-MRSA infection (62%) compared with CA-MRSA (39%). In contrast, Monecke *et al.* demonstrated high prevalence of *PVL*-positive (54%) samples in strains considered CA-MRSA in Riyadh [28]. This variation is most likely the result of the data and samples in this thesis being obtained from a hospital-based survey and not a population-based study.

All ST80 strains detected here tested positive for *PVL* gene. Moreover, all ST80 strains tested positive for *PVL* gene. Similarly, *PVL* positivity has been detected in MRSA ST 80 genotype in many countries [82-88]. Although Bin Nejam and colleagues demonstrated that all MRSA ST80 Tunisian isolates were intermediately susceptible to fusidic acid [82], all of our MRSA ST-80 strains were resistant to fusidic acid. This variation probably reflects differences in antibiotic use between the countries. Fucidic acid is commonly used for local treatment and is one of the antimicrobial agents used for elimination of MRSA in hospitals in Saudi Arabia.

The proportion of HA-MRSA infections (50%) was higher than that of CA-MRSA infections (25%) in this thesis. Similarly, Eed EM *et al.* observed in a recent study that HA-MRSA infections were more common than CA- MRSA infections in Taif [74]. However, the higher prevalence of HA-MRSA compared with CA-MRSA in this thesis probably does not reflect the true values, because the data and samples were obtained from a hospital-based survey and not a population-based study.

No correlation was found between positivity to *PVL* and CA/HA-MRSA infections. CA-MRSA has several molecular characteristics compared with HA-MRSA, for example SCC*mec* types V and IV usually produce *PVL* and are generally susceptible to non-β-lactam antibiotics [18, 89]. However, time-based definition of CA-MRSA with the recently changing epidemiology of MRSA infections may make it difficult to differentiate between CA-MRSA and HA-MRSA infections [18]. In this thesis, *PVL*-positive samples were observed in both CA-MRSA and HA-MRSA categories. Similarly, a previous study demonstrated that global CA-MRSA outbreaks could occur in the presence or absence of *PVL* gene [18]. It is possible that CA-MRSA category infections (SCC*mec*A types I, II or III) are transferred to, and colonise, patients or hospital environments and may cause hospital infections, thus becoming HA-MRSA category. Unfortunately, there were limitations to clarifying the epidemiology of MRSA infections in Taif city in the present thesis, for reasons including: no SCC*mec*A-typing, sample numbers were very low and the data were obtained by hospital-based surveillance.

There were indications that there may be an association between MRSA ST-80 and *PVL* positivity, but not with susceptibility to antimicrobial agents. More in-depth study of a larger number of clinical MRSA ST-80 samples is required to determine whether this is a genuine association or a chance observation in the small study group included in this thesis.

Regarding HRMT, this thesis successfully confirmed the reproducibility of HRM genotyping of MRSA *spa* typing. However, despite the difference in GC content between *t*008 and *t*2770 (43.7 mol% and 41.5 mol%, respectively), these *spa* types shared the same *Tm* (80.3 °C; Figure 2A). Moreover, *t*021 and *t*311 could not be separated from each other, as they shared a *Tm* (81.0 °C) (Figure 2B), probably due to the fact that they have same 44.9 mol% of the GC content. These results are in agreement with Stephens *et al*. who could not separate two *spa* types from each other based on their *Tm [40]*.

It has been suggested that shape of the melting curves are also important in determining the *spa* types [40, 90, 91]. In this thesis, there were some minor variations in the shapes of melting curves between *t*008 and *t*2770, and between *t*021 & *t*311 (Figures 2A and B). These variations in the shape of melting curves were reproducible, but it is difficult for inexperienced users to confidently predict the correct *spa* types from these variations, which highlights the challenge in optimising HRM-based *spa* typing for the growing number of *spa* types of MRSA.

Some discrepancies in *Tm* values between this study and previous investigations by Stephens *et al.* [40, 41]and Chen *et al.* [41] were observed and are summarised in Table 10. Stephens *et al*. used Platinum SYBR-Green qPCR Super Mix-UDG (Life Technologies) on a Rotor-Gene 6000 instrument (Qiagen), while a SensiMix™ HRM (Bioline) mix with Eva-Green dye on a Rotor-Gene 6000 instrument (Qiagen) was used in this thesis. Chen *et al*. used LightCycler 480 HRM Master Mix containing ResoLight dye on a LightCyclerNano real-time PCR system (Roche) [41]. The *Tm* values both for t037 and t002 were relatively close between this study and the other two studies, suggesting that the same instrument might provide similar *Tm* values for a *spa* type and that minor variations may have been caused by different reaction mixes that contained different reporting dyes. More discrepancies in the *Tm* values between Stephens *et al*. and Chen *et al*. were observed for additional *spa* types. Therefore, different instruments and reagents (dye in the reaction mix) may result in discrepancies in the *Tm* values of a *spa* type.

In this thesis, new tools for *S. sonnei* lineage genotyping using the multiplex HRM method were successfully introduced. To confirm the reproducibility, 10 samples of different lineages were run randomly in a blind experiment, which produced comparable results. Sequencing of five SNPs also independently verified the reliability of the *Tm* data, as shown in Table 11.

Table 10: *Comparison of Tm values obtained in three independent studies*

Tm	HRM-*spa* typing by		
	Stephen *et al*, 2008	Chen *et al*, 2013	Present study, 2015
79.4			*t*1544
79.5			*t*344
79.6	*t*123		
79.7	*t*352, *t*065 *		*t*044
79.8	*t*186		*t*131
80.0	*t*190		
80.1			
80.2			*t*304
80.3			*t*2770, *t*008 **, ^
80.4	*t*437 $		*t*138
80.5			
80.6	*t*127, *t*008 *		***t*037** #
80.7	*t*019 $		*t*363
80.8			*t*11986
80.9	***t*037** #, *t*1155 *		*t*018 **
81.0	*t*216		*t*311, *t*021 ^
81.1	*t*631		
81.2			***t*002** #
81.3	*t*018 **		*t*4573
81.4			*t*020
81.5			
81.6	***t*002** #		
81.7			
81.8			*t*4291
81.9			*t*032 ***
82.0			
82.1		*t*9469	
82.2	*t*202		
82.3		*t*1081	
82.5		*t*9377	
82.6		*t*4677	
82.9		*t*701	
83.1		*t*437 $	
83.2		*t*121	
83.3		*t*019 $	
83.6		***t*037** #	
84.0		*t*032 ***	
84.1		***t*002** #	
84.3		*t*9970	

**Tm* cannot distinguish *spa* types in Stephen *et al*. ***Tm* discrepancy of *spa* types between this thesis and Stephen *et al*. ****Tm* discrepancy of *spa* type between this thesis and Chen *et al*. $*Tm* discrepancy of *spa* types between Stephen *et al* and Chen *et al* studies. #*Tm* discrepancy in the three studies (text in bold).

^Melting curve shape can distinguish *spa* types in this thesis.

Table 11. *Single nucleotide polymorphisms (SNPs) used for multiplex-HRM Shigella sonnei typing*

Gene	SNP position	Nucleotide in:						
		Ancestor	Derived sequence	Lineage I	Lineage II	Lineage IIIa	Lineage IIIb	Lineage IIIc
kduD	3155111	T	C	G	T	T	T	T
deoA	4803842	G	A	G	A	G	G	G
emrA	2973337	T	C	C	C	T	T	T
fdX	2760031	G	A	G	G	G	A	A
menF	2455693	A	G	A	A	A	A	G

6 CONCLUSIONS

- The basic SHEA/ISDA practice recommendations provide an effective prevention model for reduction of CLABSI in hospital ICUs
- Multidrug-resistant *Staphylococcus aureus* strains with the *PVL* gene are circulating within hospitals in Saudi Arabia, highlighting an urgent need for continuous active surveillance and implementation of prevention measures.
- High-resolution melting has good reliability as a method for MRSA *spa* typing and *Shigella sonnei* genotyping, providing accurate, rapid and cost-effective diagnosis for epidemiological purposes. The method can be performed in diagnostic laboratories locally and globally.

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